A Salmonella Phage-P22 Mutant Defective in Abortive Transduction

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ABSTRACT

In the course of a lytic infection the Salmonella phage P22 occasionally encapsulates bacterial DNA instead of phage DNA. Thus, phage lysates include two classes of viral particles. Phage particles carrying bacterial DNA are referred to as transducing particles and deliver this DNA to a host as efficiently as particles carrying phage DNA. Once injected, the transduced DNA can either recombine with the recipient chromosome to form a "complete" transductant, or it can establish itself as an expressible, nonreplicating genetic element and form an "abortive" transductant. In this work, we describe a P22-phage mutant with reduced ability to form abortive transductants. The mutation responsible for this phenotype, called *tdx-1*, was found as one of two mutations contributing to the high-transducing phenotype of the P22-mutant HT12/4. In addition, the *tdx-1* mutation is lethal when combined with an *erf-am* mutation. The *tdx-1* mutation has been mapped to a region of the P22 genome that encodes several injected proteins and may involve more than one mutant locus. The phenotypes of the *tdx-1* mutation suggest that the Tdx protein(s) normally assist in the circularization of the P22 genome and also contribute to the formation of DNA circles thought to be required for abortive transduction.

As a generalized transducing phage, the Salmonella phage P22 occasionally packages a fragment of the bacterial genome into a P22 capsid and can deliver that DNA to a recipient cell (LEDERBERG *et al.* 1951; CASJENS and HAYDEN 1988). Two well characterized outcomes of the transduction event are known as "complete" and "abortive" transduction (Figure 1). Complete transduction refers to the substitution of donor DNA for homologous host sequences by the host recombination system (EBEL-TSIPIS *et al.* 1972a,b). During the course of recombination, part of the transduced DNA fragment is degraded; the surviving DNA is incorporated into the recipient chromosome as a double-stranded molecule. This process of homologous recombination results in stable inheritance of the transduced sequences; the acquired sequences are replicated and transmitted to both daughter cells at division.

Abortive transduction is a form of unstable genetic inheritance whereby the transduced DNA evades degradation and recombination. Abortively transduced DNA can be expressed but remains extra-chromosomal, immune to the host recombination apparatus and is not replicated. Because abortively transduced DNA is not replicated, it is inherited by only one of the two daughter cells at division (STOCKER *et al.* 1953; LEDERBERG 1956; STOCKER 1956; EBEL-TSIPIS *et al.* 1972a). Abortive transduction occurs ~10-fold more frequently than complete transduction. However, the ratio of abortive to complete transductants is observed to vary widely depending on the selected marker and the genotype of the transducing phage (SCHMIEGER 1972).

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Abortive transduction was initially described for the inheritance of flagellar characteristics and was later shown to apply to many other characteristics (OZEKI 1956; HARTMAN *et al.* 1960). Although generalized transduction was first described for the *S. typhimurium-P22* system, many other transducing phage systems are known; in particular the P1 transducing phage of *Escherichia coli* shares many features with the *S. typhimurium-P22* transducing phage (OZEKI and IKEDA 1968).

The phenomenon of P22-generalized transduction can be best understood in the context of general P22 biology (reviewed by SUSSKIND and BOTSTEIN 1978; POTEETE 1988). The P22 capsid contains a linear, double-stranded 43.4-kb DNA molecule (CASJENS and HAYDEN 1988). The capsid includes the products of genes *1, 4, 5, 7, 9, 10, 16, 20* and *26*. The majority of these proteins are essential for the assembly, structural integrity or stability of the mature phage particle. However, the products of genes *7, 16* and *20* are not essential for any of these processes; rather, these proteins are believed to be essential for the injection and/or protection of injected DNA and are probably coinjected with the DNA into the recipient host cell (HOFFMAN and LEVINE 1975a,b; ISRAEL 1977).

The size of an encapsulated DNA molecule is 43.4 kb, while a unit P22 genome is 41.8 kb in length. Because phage DNA is packaged from a concatemeric substrate, the packaged phage genome possesses repetitious ends ~1.6 kb in length (CASJENS and HAYDEN 1988). These repetitious ends are essential for P22 viability because they are the substrates for an essential, homologous recombination event that circularizes the injected P22 genome. If the P22 genome is not circular-

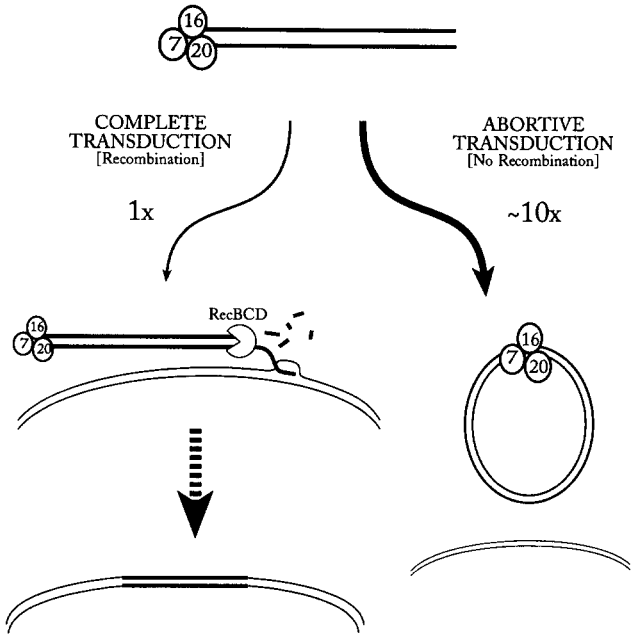

FIGURE 1.—The fate of P22-derived transducing DNA. Once transducing DNA has entered the cell it can undergo degradation and/or recombination with homologous recipient sequences (complete transduction, shown on the left). More frequently the DNA is abortively transduced (shown on the right). Details are discussed in the text.

ized, the phage will be unable to replicate its DNA and thus will not propagate. The circularization reaction is most efficiently catalyzed by the P22-encoded Erf protein, although a slightly less efficient Erf-independent pathway exists that depends on the host RecA protein (BOTSTEIN and MATZ 1975).

Once circularized, P22 DNA begins bidirectional theta replication and then switches to rolling-circle replication. Rolling-circle replication creates long concatemers of P22 genomes that are the substrates for packaging. A complex of P22 gene products *2* and *3* recognizes a site within gene *3* called "*pac*" and cleaves near *pac* to begin a processive packaging "series".

The packaging process also results in the formation of generalized transducing particles. The most plausible mechanism for this is the hypothesis that the gp2/3 complex occasionally recognizes a "pseudo" *pac* site in the bacterial chromosome and initiates a packaging series near that site. Evidence in support of this hypothesis is that (1) deletions in the host chromosome can affect the packaging of nearby host DNA (CHELALA and MARGOLIN 1974) and (2) phage mutations that increase transducing ability alter gp3 and change the DNA-sequence specificity of packaging.

While the origin of transducing particles can be viewed as the result of phage packaging functions gone "astray," it is unclear whether the fate of transduced DNA (complete or abortive transduction) is influenced by phage functions. Host functions clearly affect the fate of transduced DNA, since the RecA and RecBCD proteins dominate transductional recombination in the wild-type host (EBEL-TSIPIS *et al.* 1975a,b; MIESEL AND ROTH 1994).

Abortively transduced fragments are immune to the host recombination apparatus; it is unknown whether this immunity is provided by phage or host functions. In this work we describe the genetic analysis of a phage mutation, *tdx-1*, that eliminates the formation of abortive transductants and implicates injected phage proteins in the formation of abortive transductants. The *tdx-1* mutation is also implicated in the circularization of the phage genome by the host-dependent RecA pathway of recombination.

TABLE 1

Bacteriophage-P22 strains used in this study

Phage	Genotype	Source
MS57	Wild type	M. SUSSKIND
MS139	*5-am(N114); h21*	M. SUSSKIND
MS525	*c1-7*	M. SUSSKIND
MS607	*HT12/4; h21; int-3*	M. SUSSKIND
MS1050	*sieA44; ant-am; R222AP68*	M. SUSSKIND
MS1212	*20-am(H1030); c1-7; h21*	M. SUSSKIND
MS1379	*16-am(H1215); c1-7; h21*	M. SUSSKIND
NBP20	*erf-am(H1173); c1-7; h21*	T. POTEETE
NBP40	*tdx-1*	This work
NBP43	*5-am(N114)*	This work
NBP51	*HT12/4A*	This work
NBP52	*tdx-1; HT12/4A*	This work
NBP54	*c1-7; 20-am(H1030)*	This work
NBP63	*c1-7; 5-am(N114)*	This work
NBP80	*c1-7; HT12/4A*	This work
NBP95	*c1-7; tdx-1*	This work
NBP105	*erf-am(H1173)*	This work
NBP120	*c2-5*	This work
NBP148	*c1-7; 7-am(H1205)*	This work
NBP149	*c1-7; 16-am(H1215)*	This work
NBP179	*c1-7; tdx-1; erf-am(H1173)*	This work
NBP181	*erf-am(H1173); tdx-1*	This work
NBP183	*c1-7; erf-am(H1173)*	This work
NBP187	*c1-7; erf-am(H1173); 20-am(H1030)*	This work
NBP188	*c1-7; erf-am(H1173); 16-am(H1215)*	This work
UC754	*c1-7; HT12/4A*	S. CASJENS

NPB strains are from the collection of NICK BENSON; MS from MIRIAM SUSSKIND; UC from SHERWOOD CASJENS; DB from DAVID BOTSTEIN.

MATERIALS AND METHODS

Bacteria, phage, and general genetic methods: All phage strains are listed in Table 1. Unless specifically stated, all bacterial strains are derivatives of strain DB7000 (derived from LT2; Table 2). General phage and bacterial techniques are described by DAVIS *et al.* (1980). Routine transductions were performed with phage MS2104, kindly provided by M. SUSSKIND, as described by BENSON and GOLDMAN (1992). Many of the phage used in this work contained the plaque-morphology markers *h21* and *m44* when received. These markers were removed from phages described here using standard genetic techniques.

Media: Luria-Bertani broth (LB) was used as a rich me-

TABLE 2

Strains of *S. typhimurium* used in this study

Strain	Genotype	Source
DB7000	*fels2⁻; leuA414*(am)	M. SUSSKIND
MS1363	*fels2⁻; leuA414*(am)*; supE(gln)*	M. SUSSKIND
TT17615	*fels2⁻; leuA414*(am)*; fliD5055::*Tn10*d*Tet	BENSON and ROTH (1994)
TT18639	*fels2⁻; leuA414*(am)*; supE(gln); cob-236::*Tn10*d*Tet	This work
TT18640	*fels2⁻; leuA414*(am)*; recA1; supE(gln)*	This work
TT18641	*fels2⁻; leuA414*(am)*; cob-236::*Tn10*d*Tet	This work
TT18642	*fels2⁻; leuA414*(am)*; recA1*	This work
TT18643	*fels2⁻; leuA414*(am)*; P22(sieA44; ant-am; R222AP68)*	This work
TT18644	*fels2⁻; leuA414*(am)*; CRR299; P22(sieA44; ant-am; R222AP68)*	This work
TT18645	*fels2⁻; leuA414*(am)*; fliD5055::*Tn10*d*Tet*; P22(sieA44; ant-am; R222AP68)*	This work
TT18646	*fels2⁻; leuA414*(am)*; supE; P22[wild-type]*	This work
TT18647	*fels2⁻; leuA414*(am)*; supE; P22[erf-am(H1173) tdx-1)];*	This work
TT18648	*fels2⁻; leuA414*(am)*; supE; P22[tdx-1]*	This work
TT18649	*fels2⁻; leuA414*(am)*; P22[tdx-1]*	This work
TT18650	*fels2⁻; leuA414*(am)*; P22[wild-type]*	This work
TT18651	*fels2⁻; leuA414*(am)*; P22[erf-am(H1173) tdx-1]*	This work
TT18652	*fels2⁻; leuA414*(am)*; supE; P22[erf-am(H1173)]*	This work
TT18653	*fels2⁻; leuA414*(am)*; P22[erf-am(H1173)]*	This work

DB strains are from the laboratory of DAVID BOTSTEIN; MS strains are from MIRIAM SUSSKIND; TT strains are from JOHN ROTH.

dium and E medium as minimal medium; LB, lambda and green indicator plates are described by DAVIS *et al.* (1980). Bochner plates, used for the isolation of tetracycline-sensitive colonies, are described by BOCHNER *et al.* (1980) and made as described in BENSON and GOLDMAN (1992). Concentrations of drugs used in LB plates are as follows: kanamycin (monosulfate), 50 μg/ml; chloramphenicol, 40 μg/ml; ampicillin (Na salt), 50 μg/ml; tetracycline(HCl), 10 μg/ml. For minimal media these concentrations were reduced twofold with the exception of kanamycin, which remained the same. Motility plates are LB plates with the concentration of agar reduced fivefold. Transduction broth (TB) is LB broth supplemented with 1× E-salts and 0.2% glucose.

Phage lysates: A fresh overnight culture of the host bacterial strain was diluted 10-fold into transduction broth (TB) and a single plaque of phage was added. The culture was shaken overnight at 25° or 30°. This overnight culture was shaken with 1–3 ml of chloroform for several minutes and the chloroform allowed to settle. The TB medium was decanted from the chloroform and the cell debris pelleted by low speed centrifugation. The TB was vortexed with 0.4 ml of chloroform. At this stage P22-tail protein was added (if required) and the lysate incubated at 37° for several hours or at room temperature overnight. Lysates were stored at 4°.

Source and isolation of the *tdx-1* phage: The original source of the *tdx-1* mutation is the original mutant phage, HT12/4, isolated by SCHMIEGER (1972). Our immediate source of phage HT12/4 and therefore the *tdx-1* allele is phage MS607. Phage MS607 was provided by MIRIAM SUSSKIND as an *int-3 h21* derivative of the HT12/4 mutant. The *int-3* and *h21* mutations were removed from phage MS607 by standard genetic crosses. Phage carrying the *int*⁺ allele were identified by screening their ability to lysogenize wild-type salmonella. The replacement of the *h21* allele by the wild-type allele was determined by the change in plaque morphology on green plates; *h21* mutants have a characteristic pale or yellowish halo that contrasts with the wild-type blue halo in green agar. These crosses yielded three phage types: (1) phages with the high-transducing phenotype of phage HT12/4 (NBP52), (2) phages that were unable to specify abortive transductants (NBP40) and (3) phages that specified complete and abortive transductants at a frequency higher than wild type (NBP51). These observations suggested to us that the original HT12/4 genotype might be a composite of multiple mutations.

Construction of *erf-am tdx-1* mutant phages: Doubly mutant *erf-am tdx-1* phages with and without the *c1-7* mutation (NBP179 and NBP181) were obtained from a cross of phages NPB95 (*c1-7 tdx-1*) and NBP105 (*erf-am*). Clear and turbid plaque-forming phages that failed to plaque on a *recA1* host but could plaque on a *recA1 supE44* host were classified as *erf-am*. Some of these *c1-7 erf-am* phages were defective for abortive transduction; these are assumed to carry the *tdx-1* mutation, one such phage was assigned the name NBP179. Among the *c*⁺ *erf-am* plaques formed on a lawn of MS1363 were plaques with a slightly fuzzy and faint plaque morphology. These plaques did not form abortive transductants when used in a transduction cross and were assigned the genotype *c*⁺ *erf-am tdx-1* (NBP181). All phages of the genotype *erf-am tdx-1* failed to plate on our wild-type host (described in RESULTS). The presence of the *erf-am* mutation in these mutants was confirmed by back crosses.

Construction of lysogenic strains: Phage lysates were spotted on lawns of bacteria on lambda plates. After growth at 37° overnight, the central, turbid portion of the phage spot was streaked for single colonies that were checked for resistance to P22. Lysogenic strains were identified and induced with mitomycin C as described in a later section. The genotype of the released phage was confirmed to be correct. Because the *erf(am) tdx-1* phage (NBP181) does not grow after infection of strain DB7000, the construction of a DB7000 strain lysogenic for phage NBP181 was performed as follows. (1) Strain DB7000 was grown to log phase and coinfected with phages NBP181 (M.O.I. = 10) and NBP120 (*c2–5;* M.O.I. = 10). (2) Phage adsorption was allowed for 20 min at room temperature. (3) The mixture was plated for single colonies on LB plates. (4) After 18 hr at 37° colonies were tested for P22 resistance.

Induction of lysogenic strains: Cultures of lysogenic strains were grown to 4×10^8 cells/ml in LB, mitomycin C added (final concentration 2 μg/ml) and the culture shaken overnight at 25°. Phage were harvested by adding 0.5 ml of $CHCl_3$ to the lysed cells, shaking for 5 min, decanting the aqueous

TABLE 3

Transducing titers of varous P22 lysates

Phage	Genotype	Tet^R transductants[a] Complete transductants/ PFU ($\times 10^6$)	Abortive transductants/ PFU ($\times 10^6$)	Abortive/ complete transductants
MS57	Wild type	5.24	61.6	11.8
MS607	*HT12/4; h21; int-3*	270	~6000	~22
NBP52	*tdx-1; HT12/4A*	156	~4470	~29
NBP40	*tdx-1*	14.9	2.05	0.14
NBP51	*HT12/4A*	40.2	4850	120

[a] All donor lysates were grown on TT18641 at 25° as described in MATERIALS AND METHODS as are the transduction procedures. The data is presented as Tet^R colonies formed per plaque forming unit (PFU) in the lysate. The abortive transduction data shown for MS607 and NBP52 are approximate for reasons discussed in MATERIALS AND METHODS (see also Figure 3).

(LB) layer from the chloroform and spinning out the cell debris in the aqueous layer. To this clarified-aqueous layer an excess of P22 tail protein was added followed by incubation at 37° for 4 hr. This final step is necessary because P22 lysates made by lysogen induction are known to be tail deficient (ISRAEL *et al.* 1967).

Lysogeny assay: The frequency of lysogeny was determined by growing recipient cells to 4×10^8 cells/ml and adding phage at a multiplicity of infection of 20 phage per cell. After adsorption at room temperature for 20 min, the mixture was diluted and plated on LB plates for single colonies. These plates were incubated at 37° for 18 hr (at which time colonies were visible) and then replica printed to green plates seeded with $\sim 10^9$ virulent phage (phage H5). The green replica plates were incubated at 37° for 18 hr; lysogenic colonies appeared healthy and light green on the replica while nonlysogenic colonies exhibited no growth or poor growth and were blue. The data shown in Table 6 represents the average of at least three independent experiments with at least 300 colonies scored per experiment.

Quantitative transduction assays: The recipients in these assays are lysogenic for the phage MS1050. This phage carries the *sieA44* mutation and is deleted for the *al* gene. These prophage mutations allow the lysogen to be infected and repress expression of injected phage DNA; thus, recipients carrying this prophage are not killed by superinfecting phage during the transductional cross.

The procedure for quantification of complete and abortive Tet^R-transductants is as follows. Recipient cells were grown to a density of 4×10^8 cells/ml, mixed with phage (M.O.I. = 0.5) and left at room temperature for 20 min. The cell/phage mixture was shaken for 20 min at 37° and, for the detection of complete transductants, spread on LB tetracycline plates that were incubated for 24 hr at 37° before being scored. Abortive transductants were scored by spotting 5-μl drops on LB tetracycline plates, incubating these plates for 24–48 hr at 37° and recording the numbers of minute colonies with the aid of a dissecting microscope (10× magnification). For most assays the numbers reported are the average of at least three independent determinations per lysate with at least two independent lysates assayed (at least six independent experiments). For phages NBP607 and NBP52 the abortive transduction frequency presented is the average of one determination on each of two separate lysates. As indicated in Table 3, the estimate of abortive transduction frequency by phage strains MS607 and NBP52 is considered approximate. This uncertainty is due to the fact that microscopic evaluation of the transduction plates for phages carrying both the *HT12/4A* and *tdx-1* alleles reveals significant numbers of tiny, indistinct abortive colonies that are difficult to quantitate.

Qualitative abortive transduction assays: Abortive transductants are conveniently visualized using a flagellar transduction assay. In this assay we maintain selection for the recipient's *fliD5055::*Tn10*d*Tet insertion mutation by including tetracycline in the motility agar; the recipient can grow but is not motile. Recipients that have been abortively transduced with *fliD*$^+$ DNA retain the Tet^R-phenotype and acquire motility function; thus they become motile and "swim" away from the majority of the untransduced cells. When an abortively transduced cell divides, one of the daughter cells will not receive the abortive *fli*$^+$ DNA; that daughter and its descendants will lose motility and form a stationary colony. The daughter cell that receives the abortive fragment continues to swim through the medium. The result of this process is a "trail" of colonies marking the path of the one motile daughter cell (see Figure 2). These trails are characteristic of Fli$^+$-abortively transduced cells. Complete (Fli$^+$) transductants lose the Tn10*d*Tet insertion and are Tet^S, consequently they cannot grow on the medium.

In the experiment shown in Figure 2, the recipient is strain TT18645, which carries the insertion *fliD5055::*Tn10*d*Tet and P22 prophage. The insertion makes the cell nonmotile, and the prophage minimizes killing of host cells by phage during the experiment (described above). In this experiment the same number of Fli$^+$-complete transductants were deposited on the plate for each cross. Therefore, if the ratio of abortive to complete transductants is the same for different lysates the number of trails emanating from the deposited cells should be similar.

Quantitation of Fli$^+$-complete transductants: The number of Fli$^+$-complete transductants was determined by transducing the recipient strain (TT18645) to tetracycline sensitivity (Tet^S) on Bochner plates. To be assured that the Tet^S colonies were transductants and not due to spontaneous deletions or point mutations inactivating the Tet^R determinant; at least 100 Tet^S transductants were tested for motility function. Only transductants or precise excisions of Tn10*d*Tet will be FliD$^+$. The results showed that (1) >70% of the Tet^S cells transduced with wild-type phage (MS57) were also FliD$^+$, (2) >90% of the cells transduced with NPB51 or NBP40 phage were FliD$^+$, (3) 100% of the transductants treated with NBP33 or NBP52 lysates were Fli$^+$, and (4) of the Tet^S colonies obtained from the no-phage control, 100% were Fli$^-$, suggesting that none of the Tet^S Fli$^+$ Bochner-selected colonies were due to precise excision of the Tn10*d*Tet and that all tested Tet^S Fli$^+$ colonies were in fact transductants. This experiment was also per-

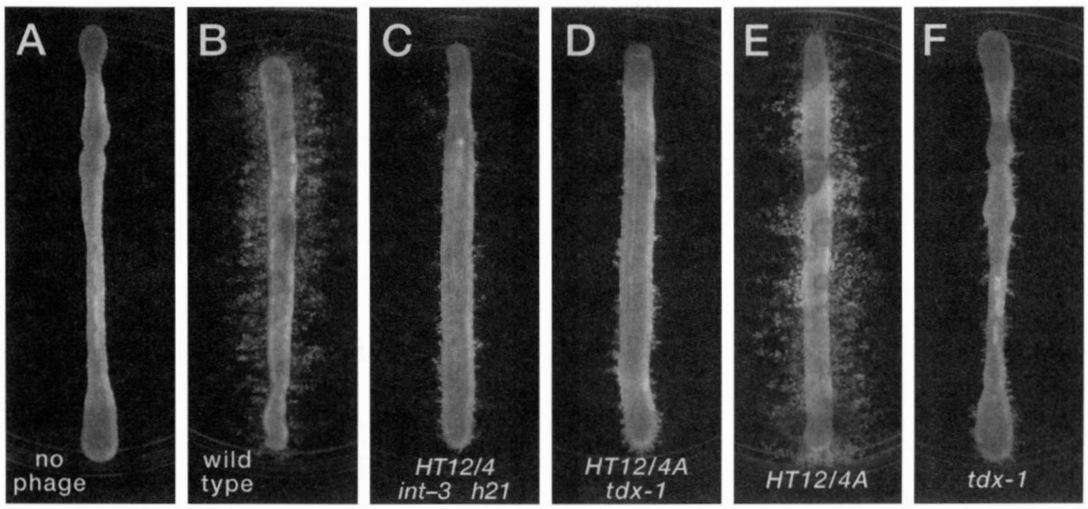

FIGURE 2.—Trails formed by the abortive transduction of Fli⁻ recipient strains. The recipient is strain TT18645. Phage donors were as follows: none (A), MS57 (B), MS607 (C), NBP52 (D), NBP51 (E), NBP40 (G). Phage genotypes are shown at the bottom of each panel. The experimental protocol is described in MATERIALS AND METHODS.

formed in parallel with the experiments shows in Figures 2 and 4 to verify the accuracy of the Fli⁺-complete transductant titers (*i.e.*, the same mixture that was plated on the swarm agar was plated on Bochner medium).

Complementation of *tdx-1* mutants by wild-type P22: The FliD⁺-abortive transduction assay was used to detect complementation of the abortive transduction phenotype of *tdx-1* mutants. Recipient cells were grown as described for the quantitative transduction assay. Wild-type helper phage was grown on strain TT17615 that cannot provide the Fli⁺ function. The M.O.I. of helper phage was 3.5, while that of phage NBP40 was 1.6. Phage were premixed, added to cells, and allowed to adsorb at room temperature for 20 min; the mixture was then deposited on motility agar containing tetracycline and the plates incubated at 37° for 24 hr.

RESULTS

Assays for abortive transduction: We employ two assays for abortive transduction. Our qualitative assay is based on the motility phenotype of abortively transduced *fli* mutants (described in MATERIALS AND METHODS). Transduction of a nonmotile (Fli⁻) strain with *fli*⁺ DNA generates complete transductants (which multiply and form confluent "swarms" throughout the motility plate) and abortive transductants (which form discrete colony trails traversing the motility plate; STOCKER 1956; LEDERBERG 1956). In the experiment shown in Figure 2, complete transductants are prevented from growing and only the nonmotile recipient and abortive transductants may grow. The presence of trails on a motility plate in this experiment is a qualitative indication of the occurrence of abortive transduction.

Our quantitative assay for abortive transduction is based on selection for inheritance of tetracycline resistance. For this assay the recipient carries a large deletion of the chromosomal region homologous to the transduced fragment, which carries the defective transposon Tn10*d*Tet. Under the conditions of the experiment no complete transductants are observed because the recipient's deletion is too large to be repaired by a single transduced fragment. The elimination of complete transductants greatly facilitates the scoring of abortive transductants.

The high-transducing phage HT12/4 is impaired for the formation of abortive transductants: The original P22 mutant HT12/4 is one of several phage mutants isolated by virtue of its increased frequency of complete transduction (SCHMIEGER 1972). While using this phage in standard transductional crosses we observed that abortive transductants were not as readily apparent as in crosses mediated by a different high transducing phage (HT105/1) or by wild-type P22. This defect in abortive transduction is seen in the motility assay depicted in Figure 2.

Figure 2 shows trails formed by abortive transduction of a recipient carrying the mutation *fliD5055*::Tn10*d*-Tet. In Figure 2B the transducing phage is wild-type P22; numerous trails (evidence of abortive transduction) are evident. In Figure 2C the donor lysate was made with phage MS607, a derivative of the original phage HT12/4 of SCHMIEGER. It is apparent that this lysate generates fewer abortive transductants (deduced by the lack of trails). For all panels in this figure, approximately equal numbers of FliD⁺ complete transductants have been deposited on each plate for each cross (see MATERIALS AND METHODS). If the ratio of complete to abortive transductants is the same for each phage, we would expect equal numbers of abortive trails in each panel. Since there are virtually no trails evident for phage HT12/4, we conclude that this phage is defective for the ability to specify abortive transductants. When a higher concentration of HT12/4-derived lysate is used, some trails do appear; thus the ability of phage HT12/4 to perform abortive transduction is impaired but not completely lost (data not shown).

Figure 3 shows an abortive-transduction assay based

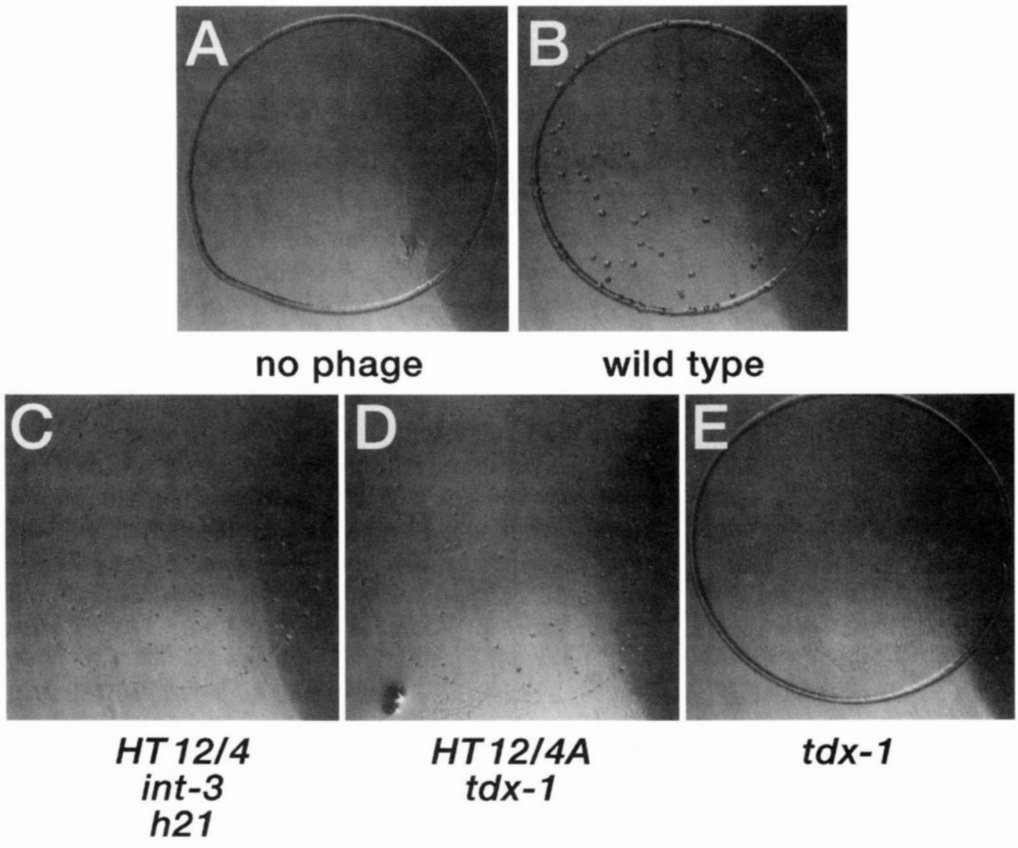

FIGURE 3.—Tetracycline-resistant abortive transductants. Donor lysates were made with the following phage: no phage (A), MS57 (B), MS607 (C), NBP52 (D), NBP40 (E). The appearance of Tet^R-abortives formed by phage HT12/4A (NBP51) is identical to wild type (data not shown). Details are given in the text.

on inheritance of Tet^R. Figure 3B shows wild-type, Tet^R-abortive transductant colonies. Figure 3C shows abortive transductants made by phage HT12/4 (MS607). In the Tet^R-based assay, phage HT12/4 still forms abortive transductants but the abortive colonies are smaller and less distinct than wild-type-Tet^R abortives. The reason for the difference between the Fli and Tet assays is unclear, but the results of the Tet assay seem significant and will be discussed later. Tet^R-abortive and complete transductants made by wild-type and HT12/4 phage are quantified in Table 3.

The genetic basis for the HT12/4-transducing phenotype: The HT12/4 phage used in the above experiments contained the *h21* and the *int-3* mutations in addition to the *HT12/4* mutation. To examine the contribution of these mutations to the HT phenotype, we performed phage crosses to replace these alleles with their wild-type counterparts (described in MATERIALS AND METHODS). These crosses generated recombinant phages that displayed transducing phenotypes not seen for either of the parent phages.

Transduction characteristics of these novel transducing phage are shown in Table 3 (see also Figures 2 and 3). One class of phage, assigned the genotype *tdx-1,* shows slightly increased complete transduction (two-fold, compared to wild-type) and a deficiency in the formation of abortive transductants (Figure 2F; Figure 3E; Table 3). The second class of transducing phage, assigned the genotype *HT12/4A,* is 10-fold elevated for the formation of complete transductants and makes an excess of abortive transductants compared to wild-type phage (Table 3; Figure 2). Since the above phages lack the *int* and *h21* alleles, we conclude that these mutations have no role in the transducing phenotype of phage HT12/4.

We interpret these data to indicate that the HT12/4 phenotype is due to both the *tdx-1* and *HT12/4A* mutations. This hypothesis was confirmed by the following cross. Phage NBP52 (*HT12/4A tdx-1*) was crossed with phage NBP63 (*c1–7 5-am*). Recombinant *c1–7* 5^+ phages were isolated and their transducing phenotypes analyzed. Again, recombinant phages with either the *tdx-1* or the *HT12/4A* genotypes were recovered (data not shown). Thus, we conclude that the HT12/4 phenotype reflects the combined effects of at least two separable mutations that we designate *tdx-1* and *HT12/4A.*

CASJENS *et al.* (1992) have cloned gene *3* of phage HT12/4 and demonstrated that the single base-pair mutation present in gene *3* of phage HT12/4 is necessary and sufficient to endow P22 with the HT phenotype and altered *pac*-site specificity characteristic of phage HT12/4 (JACKSON *et al.* 1982; CASJENS *et al.* 1992). These authors remarked in their work that their reconstructed *HT12/4A* phage showed altered transduction properties compared to the original *HT12/4* phage. We have compared the quantitative transducing phenotype and *pac*-site specificity of CASJENS *et al.*'s reconstructed *HT12/4A* phage with the phage we have designated

TABLE 4

Efficiency of plating P22 strains harboring different combinations of *erf* and *tdx* alleles on Salmonella strains with different *recA* and *supE* alleles

Phage	Phage genotype	Efficiency of plating on different hosts[a]			
		Wild type	*supE*	*recA1*	*recA1 supE*
NBP85	*c1-7; tdx-1*	0.95	1	0.88	0.83
NBP183	*c1-7; erf(am)*	0.29	1	1.47×10^{-7}	0.83
NBP179	*c1-7; erf(am); tdx-1*	1.41×10^{-6}	1	1.1×10^{-6}	0.76

[a] The wild-type strain is DB7000. The *supE* strain is MS1363. The *recA1* strain is TT18642. The *recA1 supE* strain is TT18640. The plating efficiencies are normalized to the titer of phage on strain MS1363. All lysates were prepared on strain MS1363 at 25° as described in MATERIALS AND METHODS. The data presented is the plaquing efficiency relative to the supE strain. Data is shown for one particular lysate, for which the value of 1 corresponds to 2.35×10^{11} (NBP85), 3.89×10^{11} (NBP183), 1.46×10^{11} (NBP179).

HT12/4A (NBP51) and found them to be identical (data not shown).

The *tdx-1* mutation is lethal in an *erf-am* background: Figure 1 describes a model for the transduction process in which an abortive transductant is formed by a protein-mediated, noncovalent circularization of the transduced DNA (SANDRI and BERGER 1980b). If this model is correct, then P22 DNA might also assume such a structure as an intermediate during the essential circularization of the phage genome. Since the P22-encoded Erf protein is known to catalyze the circularization step (BOTSTEIN and MATZ 1970; POTEETE 1988), we constructed an *erf-am tdx-1* double mutant anticipating that such a phage might show a revealing phenotype.

Phages carrying null *erf* alleles cannot circularize their genome by the Erf-mediated pathway and are dependent on the host's RecA protein for circularization. Thus Erf$^-$ phages make plaques on wild-type hosts but not on *recA* hosts. Table 4 shows the efficiency of plating *tdx-1* phages, *erf-am* phages and *erf-am tdx-1* phages on various hosts. The *erf-am tdx-1* double mutant does not plaque on our wild-type host (line 3), while phages carrying either the *tdx-1* or the *erf-am* mutation plaque efficiently on the wild-type host (lines 1 and 2). From these data we conclude that an *erf-am tdx-1* phage cannot use the host's RecA pathway of recombination, suggesting that the *tdx-1* mutation blocks the RecA-dependent pathway of circularization.

Genetic mapping of the *tdx-1* allele: The *erf-am tdx-1*—lethal phenotype allowed us to map the *tdx-1* mutation using amber mutations in a variety of essential genes in combination with an *erf-am* mutation. Preliminary cross data suggested that the *tdx-1* mutation resided near genes *7, 20* and *16* (data not shown). Table 5 shows the results of crosses between *erf-am tdx-1* phages with *erf-am 20-am* phages, or *erf-am 16-am* phages. The genetic distance between the *16-am* allele and the *tdx-1* mutation (line 5) is approximately threefold smaller than the distances separating the *20-am* and *tdx-1* alleles (line 4) or the *16-am* and *20-am* alleles (line 6). This data localizes the *tdx-1* mutation in or near gene *16*.

The abortive transduction phenotype of *tdx-1* mutant phage can be corrected by *tdx*$^+$-helper particles: When *16-am* or *16-ts* phage are grown under nonpermissive conditions they form progeny particles that lack the product of gene *16* (gp16; HOFFMAN and LEVINE 1975a). These gp16-defective particles are noninfectious but can be rendered infectious by coinfection with phage particles that contain wild-type gp16 (HOFFMAN and LEVINE 1975a,b). HOFFMAN and LEVINE showed that the DNA of the coinfecting, gp16$^+$ helper phage need not be expressed and concluded that the correcting activity must be effected by gp16 present in the helper-phage particle. If the *tdx-1* mutation affects gene *16*, as suggested by the mapping data in Table 5, then the abortive-transduction defect of *tdx-1* phages might be corrected by coinfecting, wild-type phage.

Figure 4 shows a complementation experiment utilizing a *fliD* mutant recipient. Figure 4D clearly shows that wild-type, coinfecting phage can complement the *tdx-1* donor phage for the formation of abortive transductants (compare C and D). In this experiment the helper

TABLE 5

Genetic mapping of the *tdx-1* mutation

Relevant genotypes of recombining phages[a]		% *tdx*$^+$ *am*$^+$ recombinant phage[b]
Phage 1	Phage 2	
tdx-1	—	$<10^{-5}$; $<10^{-5}$
20-am	—	$<10^{-5}$; $<10^{-5}$
16-am	—	$<10^{-5}$; $<10^{-5}$
tdx-1	*20-am*	0.29; 0.25
tdx-1	*16-am*	0.09; 0.08
16-am	*20-am*	0.27; 0.28

[a] In these crosses both phages carry the *erf-am(H1173)* and *c1-7* mutations. The *tdx-1* phage is NBP179, the *20-am* phage is NBP187, and the *16-am* phage is NBP188.

[b] The results of two independent experiments are presented. All recombinant phage carry the *erf-am* mutation. The numbers reported are the total plaque forming units determined on DB7000 (*tdx*$^+$ *20*$^+$ *16*$^+$ phage titer) divided by the titer of phage on MS1363 (all phage) multiplied by 100.

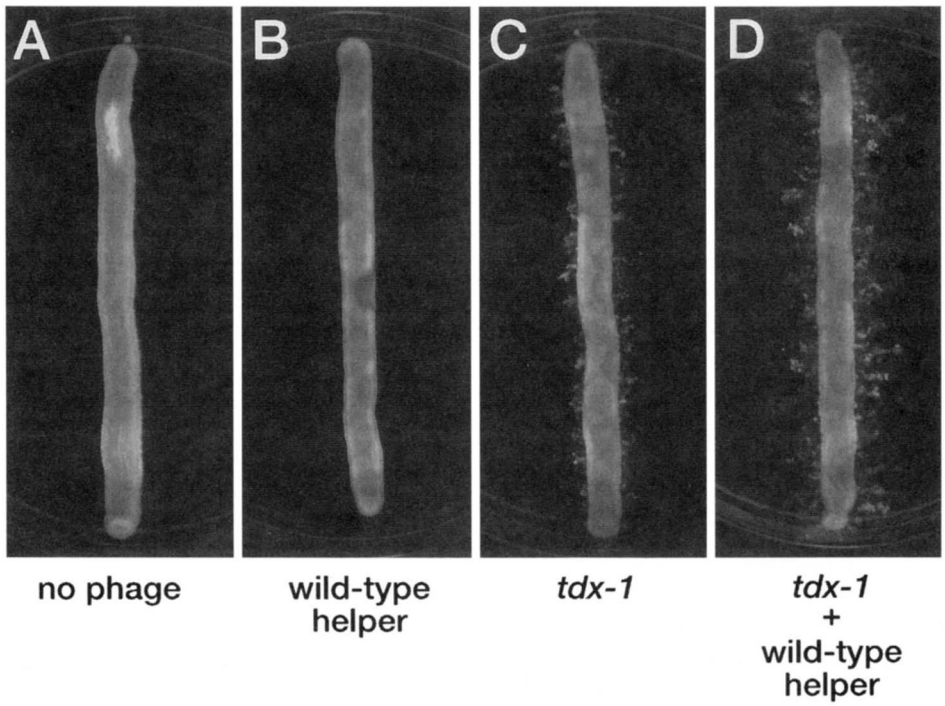

FIGURE 4.—Correcting the abortive transduction phenotype of *tdx-1* phages with wild-type helper phage. The wild-type, helper phage (NBP31) has been grown on a Fli$^-$ strain (TT17615) and cannot provide Fli function (B). The *tdx-1* phage was NBP40; the recipient in this experiment was NBP1121. A complete discussion can be found in the text.

phage was grown on a host containing the same *fli* mutation as the recipient and therefore cannot possibly contribute *fli*$^+$ sequences (Figure 4B). Furthermore, since the recipient is a lysogen, the helper phage cannot express its late genes suggesting that the Tdx$^+$ function is provided by the helper-phage capsid. Particles defective in gp16 do not provide this help (data not shown). This result is consistent with the *tdx-1* mutation residing in gene *16*. However, we have recently determined that gp20-defective particles are also helped by wild-type helper phage (and also fail to help *tdx-1;* data not shown); therefore, the outcome of the *tdx-1* correction experiment is consistent with either a mutation in gene *20* or gene *16*.

The *tdx-1* mutation affects an early step in the maturation of injected *erf-am tdx-1* phage DNA: The growth defect of *erf-am tdx-1* phages could be early or late in development. If the growth defect of *erf-am tdx-1* phages reflects a failure to circularize the injected genome (as the involvement of the *erf-am* allele implies), we would expect that this phage would be defective in both lysogeny and lysis. Alternatively, if the doubly mutant phage is defective in a later stage of lytic growth, we might expect it to lysogenize a wild-type host with wild-type efficiency.

Table 6 shows the ability of phages carrying various combinations of *erf* and *tdx* alleles to lysogenize various hosts. The data show that singly mutant *erf-am* or *tdx-1* phages can lysogenize a wild-type host with high efficiency; however, the doubly-mutant *erf-am tdx-1* phages lysogenize a wild-type host ~10-fold less frequently than the *erf-am* phage. The observation that an *erf-am tdx-1* phage retains some ability to lysogenize a *recA*$^+$ cell (6% of infected cells) compared to a *recA*$^-$ cell (≤0.4%) indicates that the *tdx-1* mutation reduces but does not eliminate circularization by the RecA pathway.

Once circularized, *erf-am tdx-1* phage exhibit wild-type lytic growth: The data in Table 6 does not rule out the possibility that *erf-am tdx-1* phages have both pre- and postcircularization defects. To determine if *erf-am tdx-1* phages have a defect in development subsequent to circularization of the phage genome, we assessed the ability of such phage to develop when the phage DNA was circularized by an Erf/RecA-independent mechanism.

An alternative means of achieving circularization is by use of the site-specific recombination system of the phage. Excision of a prophage from the host chromosome requires the P22-encoded Int and Xis proteins and the *cis*-acting sites that flank the prophage. This site-specific recombination system does not require Erf or RecA function (POTEETE 1988). If *erf-am tdx-1* phages are defective only in circularization (or an earlier metabolic step) we would predict that induction of an *erf-am tdx-1* prophage would yield an amount of phage comparable to that released by inducing a wild-type prophage.

The results presented in Table 7 clearly demonstrate that the lysates produced by mitomycin C induction of wild-type, *tdx-1*, *erf-am* or *erf-am tdx-1* prophages are nearly equal in titer, whether the induction was carried out in a *supE* or *sup*^{0} genetic background. Because an *erf-am tdx-1* phage has a wild-type burst size once it has been circularized, we conclude that *erf-am tdx-1* mutant phages are not defective for any developmental lytic step after circularization of the phage genome.

DISCUSSION

We have described a mutant of phage P22 that is defective in the formation of abortive transductants.

This mutation, *tdx-1*, was initially discovered as one of two mutations contributing to the high-transducing phenotype of the phage HT12/4. We have demonstrated the following.

1. The *tdx-1* mutation reduces the ability to form abortive transductants and slightly increases the formation of complete transductants.
2. The *tdx-1* mutation appears to map in or near P22-gene *16*.
3. The abortive transduction defect of *tdx-1*-transducing particles can be corrected by wild-type, coinfecting phage, a fact that is consistent with known phenotypes of gp16-defective particles (HOFFMAN and LEVINE 1975a,b) and gp20-defective particles (N. BENSON, unpublished data).
4. An *erf-am tdx-1* mutant phage cannot grow on a wild-type host; from which we conclude that the *tdx-1* mutation affects the RecA-catalyzed pathway of phage chromosome circularization.
5. The *erf-am—tdx-1* lethal phenotype is circumvented when the phage genome is circularized by the phage's site-specific recombination system; from this observation we conclude that the defect in *erf-am tdx-1* phage development is at or before the critical circularization stage of infection.

Our data also suggest a more complex involvement of gp3 in the formation and/or stability of abortive transductants (in addition to the packaging of DNA). First, the Tet^R-abortive transduction data presented in Figure 3 and Table 3 show that phages carrying the *tdx-1* allele (in a wild-type gene *3* background) are severely deficient for the formation of Tet^R-abortive transductants (Figure 3E). However, when the *tdx-1* mutation is accompanied by an *HT12/4A* mutation (a mutant gene *3* background), Tet^R-abortive transductants are "resurrected" (albeit weakly; compare D and E in Figure 3). Second, the ratio of abortive to complete transductants for phage HT12/4A is 120, while the ratio of complete to abortive transductants for wild-type P22 is 11.8 (Table 3). This suggests that the mutant gene *3* allele in an HT12/4A phage affects the formation of abortive transductants. Both these observations are consistent with the mutant gp3 (specified by the *HT12/4A* mutation) being packaged in the phage capsid. This conclusion is surprising since gp3 has not been found as a component of the capsid. However, it is possible that a few molecules of gp3 might be present in the capsid and escape detection (S. CASJENS, personal communication).

TABLE 7

Titers of lysates made by inducing lysogens of varying prophage genotype

Phage	Prophage genotype	Titer of lysates made by induction of wild-type and *supE* lysogenic hosts[a]	
		Wild-type (sup^0) host ($\times 10^{-10}$)	*supE* host ($\times 10^{-10}$)
NBP31	Wild type	9.7	3.4
NBP40	*tdx-1*	8.2	3.8
NBP105	*erf-am*	18	4.5
NBP181	*tdx-1; erf-am*	10	2.4

[a] Lysogenic strains are as follows: wild type, TT18650, 1187, 1195 and 1193 (top to bottom); *supE*, TT18646, 1186, 1194 and 1185. Numbers shown are the average of two independent induction experiments. The titer of the lysates was determined on MS1363 (*supE*).

The *tdx-1* mutation maps to a region of the P22 genome that encodes proteins carried in the mature viral particle. We have attempted to assign the *tdx-1* mutation to a precise locus with several experiments. When *erf-am tdx-1* phages are crossed with a plasmid containing the P22 *Eco*RI-B fragment (9.28 kb; JACKSON *et al.* 1978), *erf-am* tdx^+ progeny phages are recovered. The *Eco*RI-B fragment extends from the middle of gene *8* through gene *16* (the *Eco*RI site is 40 nucleotides past the end of gene *16*). However, when *erf-am tdx-1* phages are crossed with a minimal clone of gene *16* (pBU5; UMLAUF and DREISEIKELMANN 1992) no *erf-am* tdx^+ phages are recovered, nor are such recombinants seen with crosses using clones that include genes *16* and *20* (provided by PETER BERGET; data not shown). Furthermore, all spontaneous reverent phages (those plating on a wild-type host) are genotypically erf^+ *tdx-1* and never *erf-am* tdx^+ (data not shown). These experiments, taken in consideration with the mapping data in Table 5 and the complementation data in Figure 4, suggest that a locus in addition to genes *16* or *20* may be involved.

TABLE 6

The ability of phage carrying different *erf* and *tdx* alleles to lysogenize $recA^+$ and *recA1* hosts

Phage	Genotype	% lysogens formed on various hosts[a]			
		Wild type	*supE*	*recA1*	*supE recA1*
NBP105	*erf(am)*	58	95	≤0.3	59
NBP40	*tdx-1*	100	100	87	85
NBP181	*erf(am); tdx-1*	6	≥99.7	≤0.4	42

[a] Recipient strains are DB7000 (wild type), MS1363 (*supE*), TT18642 (*recA1*), TT18640 (*recA1 supE*). The numbers shown are the percentage of surviving colonies that are lysogens.

We have also attempted to complement the Tdx-1 phenotype with the plasmid pBU5, which produces gp16 under the control of the Lac repressor. Strains harboring pBU5 cannot rescue *erf-am tdx-1* phage although these strains can rescue *16-am* phage (in a plating assay; data not shown). Furthermore, growing *tdx-1* mutant phage (NBP40) through a gp16 (pBU5) producing strain does not enable the progeny phage to specify abortive transductants nor does plating *tdx-1*-transducing particles on a host expressing gp16 effect an observable increase in abortive transductants (data not shown). We conclude that gp16 alone cannot rescue the Tdx-1 mutant phenotypes. These observations reinforce our belief that the Tdx-1 phenotypes are due to the combined effects of mutations in genes *16* or *20* and an additional, unidentified locus.

We have been unable to unambiguously assign the *tdx-1* mutations to known P22 loci, nonetheless, several conclusions can be drawn. The fact that the *tdx-1* mutations affect generalized transducing particles and can be corrected by coinfecting phage (Figure 4) suggests that the Tdx^+ proteins are carried within the viral particle and are injected with DNA into the new host. Thus, the identity of Tdx^+ must be restricted to the injected components of the mature P22. This narrows Tdx^+ to the products of genes *7, 16, 20* or *26;* all of which encode proteins believed to be injected with the DNA (ISRAEL 1977).

In considering the role of injected phage proteins, it is useful to review early events in phage infection. Injected phage DNA must cross the outer membrane, the periplasmic space and the inner membrane to reach the cytoplasm. Unlike some T-phages, the architecture of the P22 particle does not seem to allow for direct phage penetration of the membranes and injection into the cytoplasm (DREISEIKELMANN 1994). Rather, it seems likely that phage (and transducing) DNA must first enter the periplasmic space and then traverse the inner membrane.

Once inside the cytoplasm, the double-stranded DNA must evade the RecBCD nuclease that can degrade the phage nucleic acid. During the later stages of infection the P22 genome avoids RecBCD-catalyzed degradation by circularizing and then by guiding synthesis of the phage Abc1 and 2 proteins. These proteins physically inhibit the RecBCD-catalyzed degradation of the linear, concatemeric DNA from which infectious particles are made (POTEETE 1988). It is not known how P22 DNA escapes RecBCD-catalyzed degradation of DNA before circularization; sequestration of the blunt ends by injected phage proteins is a likely possibility. We propose that injected phage proteins protect DNA ends and promote genome circularization (and may also help in transport across the membrane as discussed below). Specifically we suggest that formation of abortively transduced fragments requires the presence of these proteins at DNA ends. Normally these proteins assist in the circularization of a phage genome that possesses homologous, direct repeats at each end. Since transduced fragments do not possess such repeats, the circularization process is aborted leaving a stable, protein-held DNA circle (an abortively transduced fragment).

The proposal that an abortive transductant is formed by circularization of a transduced DNA fragment was suggested for the transducing phage P1 by SANDRI and BERGER (1980). These authors showed that the mobility of P1-abortively transduced DNA in native gels or neutral sucrose gradients was characteristic of a circular molecule. When this suspected circular DNA was purified and subjected to denaturing conditions, or exposed to Pronase, the DNA assumed the mobility characteristic of full-sized, linear P1-particle DNA. Given the many parallels between P1 and P22 transduction (OZEKI and IKEDA 1968), it is likely that these phages share a similar mechanism for abortive transduction. The *tdx-1* mutant characterized here is an excellent candidate for a defective P22 analogue of the P1-circularization apparatus.

Our model for P22 transduction (Figure 1) incorporates the hypothesis of SANDRI and BERGER (1980) that abortive transduction is due to protein mediated, noncovalent circularization of the transduced DNA. Figure 1 shows a complex of gp7/20/16 at one end of the DNA fragment (it is possible that both ends are protected; see BENSON and ROTH 1994). We suggest that the eventual fate of the transducing fragment is determined by a competition for the unprotected end by the protected end (resulting in a circle and abortive transduction) and RecBCD enzyme (resulting in degradation and recombination). Circle closure is usually the winner in this competition, a fate that makes sense for the phage genome because this protects against RecBCD degradation. Thus, we visualize abortive transductants as a trapped intermediate of a circle-closure reaction; phage DNA transiently assumes this form before progressing to a recombinant, closed circle.

There are genetic and physical data relevant to this model. HOFFMAN and LEVINE (1975a,b) have conducted an extensive analysis of gene *16* mutations and their effect on the fate of injected DNA. These authors concluded that gp16 did not protect injected DNA from host degradation but might be involved in some aspect of the translocation of DNA across the cell membrane into the intracellular space. UMLAUF and DREISEIKELMANN (1992) have cloned gene *16* and shown that (1) gp16 expressed in *E. coli* cannot rescue gene *2* mutants of bacteriophage T4 (*i.e.*, cannot protect naked T4-DNA ends), (2) that gp16 expressed from a plasmid in Salmonella cannot rescue the DNA from gp16-defective P22 particles, and (3) that *recD* mutations (which inactivate the RecBCD nuclease activity) do not increase the plaquing efficiency of P22 *16-am* mutants. These results, taken together with those of HOFFMAN and LEVINE (1975a,b), would indicate that gp16 alone does not pro-

tect blunt, double-stranded DNA ends; a conclusion consistent with our data.

The data discussed above lead us to hypothesize that translocation and protection of injected DNA are effected by the coordinated actions of at least gp7/20/16, probably in a complex. The data presented here, in addition to our unpublished data, suggests that the Tdx-1 phenotype is effected by a minimum of two mutations, one of which we believe to lie in either gene *16* or *20,* and another located within the boundaries of the *Eco*RI-B fragment. We suggest that the *tdx-1* mutations affect the wild-type proteins in such a way that noncovalent circle closure (abortive transduction) is impaired but that DNA transport and protection activities are conserved.

In summary, we have described a mutant phage that is defective in both the phenomenon of abortive transduction and the RecA-dependent circularization of the P22 genome. We suggest that the phenomenon of abortive transduction is best described as a futile attempt by injected P22 proteins to circularize transduced DNA.

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